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Acute effects of intravenous heroin on the hypothalamic-pituitary-adrenal axis response: A controlled trial

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Abstract

Heroin dependence is associated with a stressful environment and with dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis. The present study examined the acute effects of intravenous heroin versus placebo on the HPA axis response in heroin-dependent patients.

Twenty-eight heroin-dependent patients in heroin-assisted treatment (HAT) and 20 age and gender matched healthy participants were included in a controlled trial in which patients were twice administered heroin or saline in a crossover design, and healthy controls were only administered saline. The HPA axis response was measured by adrenocorticotropic hormone (ACTH) levels and by cortisol levels in serum and saliva before and 20 min and 60 min after substance administration. Craving, withdrawal and anxiety levels were measured before and 60 min after substance application. Plasma concentrations of heroin and its main metabolites were assessed using high-performance liquid chromatography.

Heroin administration reduces craving, withdrawal and anxiety levels and leads to significant decreases in ACTH and cortisol concentrations ($p<0.01$). After heroin administration, cortisol concentrations did not differ from healthy controls, and ACTH levels were significantly lower ($p<0.01$). In contrast, when patients receive saline, all hormone levels were significantly higher in patients than in healthy controls ($p<0.01$).

Heroin-dependent patients showed a normalized HPA axis response compared to healthy controls when they receive their regular heroin dose. These findings indicate that regular opioid administration protects addicts from stress and underscore the clinical significance of HAT for heroin-dependent patients.

Key-words:

Addiction; Cortisol; Diacetylmorphine (DAM); Heroin-assisted treatment (HAT); Heroin dependence; Hypothalamic-pituitary-adrenal (HPA) axis

Introduction

Substance dependence is a chronic relapsing brain disorder that is characterized by an overwhelming compulsion to seek and use drugs, despite negative consequences (1). It is well known that substance dependence is marked by abnormal hypothalamic-pituitary-adrenal (HPA) axis function (2, 3). An atypical stress response occurs in both heroin and cocaine dependence (4-6). Whereas cocaine activates the HPA axis and thus elevates adrenocorticotropic hormone (ACTH) and cortisol levels (7), heroin and other opiates may suppress stress hormone secretion (8, 9). HPA axis activation has been observed during opioid withdrawal syndromes (10, 11).

Stress and stress response are closely associated with drug use. Stress is known to increase drug craving, anxiety, ACTH and cortisol secretion and may be associated with further drug use (12-15).

It has been argued that heroin-dependent patients suffer from persistent hyper-responsiveness to stress, even after detoxification, which reflects heightened sensitivity of the hypothalamus and the pituitary gland to negative emotional stimuli and consequently might contribute to later drug use (16).

Maintenance treatment with methadone or buprenorphine is seen as the treatment of choice in heroin dependence (17). Alternative pharmacological strategies have been considered as treatment options, including the prescription of heroin (diacetylmorphine, DAM) itself. Heroin-assisted treatment (HAT) is now available in several countries and has given good outcomes (18).

Regular opioid administration could damp the inadequate stress response of heroin-dependent patients and thus defend the individual from aversive experiences such as negative affects (19).

There is empirical evidence that the altered HPA axis function in heroin dependence partially returns to normal during opioid maintenance treatment (20, 21). However, some abnormalities in the HPA axis response seem to persist even during stable opioid maintenance treatment (22, 23).

This study examined the acute effects of heroin on the HPA axis response in heroin-dependent patients, in comparison to placebo and to healthy participants. For ethical reasons, healthy controls

were included for placebo administration only. We hypothesized that HPA axis activity would be reduced after heroin administration and that the HPA response in heroin-dependent patients would be normalized in comparison to healthy participants.

Patients and methods

Study sample

Table 1 summarizes the socio-demographic and diagnostic characteristics of the study sample. Twenty eight heroin-dependent outpatients were recruited from the Division of Substance Use Disorders of the Psychiatric Hospital of the University of Basel (Switzerland). They were aged 23-58 years (mean age = 41.3, SD = 6.6), met the DSM-IV diagnostic criteria for opioid dependence and had been in heroin-assisted treatment for 6.7 years on average (SD = 4.5). Their daily dose of prescribed heroin ranged from 30 mg to 700 mg per day.

The inclusion criteria were: age older than 18 years, history of intravenous heroin dependence, having been on current stable heroin-assisted treatment for at least 6 months, and unchanged heroin dose during the previous 3 months. Exclusion criteria were a positive alcohol breathalyzer test and an additional physical disease or psychiatric disorder, including other comorbid substance dependence.

In the heroin-assisted treatment regime, heroin is administered twice a day. The details of the heroin-assisted treatment program in Switzerland have been described elsewhere in more detail (24, 25).

When patients fulfilled the inclusion criteria, the history of heroin and other illicit substance use was assessed with the semi-structured interview according to ICD-10 research criteria. Patients were told to abstain from illicit drug use other than prescribed heroin for the duration of the study, from alcohol intake for 72 hours and from tobacco consumption for 2 hours before scanning. Before the experiment, the patients had had no opioid intake for about 10 hours.

The healthy controls were carefully screened using a semi-structured clinical interview to exclude psychiatric or physical illness or a family history of psychiatric illness. Participants who had ever used any other illicit psychotropic drug, who consumed more than 20 g alcohol per day, or who had any psychiatric, neurologic, or severe medical illness history, were also excluded. Healthy controls were recruited from the general population by advertisement in the same geographical area. After the study had been completely described to the subjects, written informed consent was obtained. The study was approved by the local ethics committee.

[Please here Table 1]

Study design

The heroin-dependent patients were examined on two occasions one week apart, in a crossover design. The patients were randomly assigned following simple randomization procedures (computerized random numbers) to one of two injected substance (heroin vs. placebo = saline). One patient group (n = 14) received first heroin before the experiment, whereas the second patient group (n = 14) received first saline. One week later, they received the other substance before the experiment. All patients were informed that they received heroin or placebo before or after the experiment. The patients and the experimenter were blind to the administered substances. The healthy controls participated only in the placebo condition. The study has been registered by the website http://clinicaltrials.gov (ID NCT01174927).

Study procedure

The study procedure is shown in Table 2. At the start of the study day, a urine sample was collected for screening for amphetamines, benzodiazepines, cocaine, methamphetamine, morphine, and THC using immunometric assay kits. Alcohol use was tested with an alcohol breathalyzer test.

After completion of the baseline measurement, heroin-dependent patients received either their dose of prescribed heroin in 5 ml, or the same dose of saline, through an indwelling intravenous catheter over a period of 30 sec. Healthy controls were injected with 5 ml of saline over 30 sec. Heroin was provided by the Swiss Federal Office of Public Health in the form of the hydrochloride salt. It was dissolved in sterile water on site and aspirated into a syringe, adapted to the evacuated infusion system (26).

Adrenocorticotropic hormone (ACTH) and cortisol were taken as measures of the HPA axis response. Samples were collected through an intravenous catheter at baseline, 20 min and 60 min after substance administration. The concentrations of heroin and its metabolites were obtained at baseline and 3 min, 10 min and 60 min after the patients had received their heroin injection. Self-report measures were assessed before and 60 min after substance application.

[Please here Table 2]

Bioanalytical and biochemical measurements

The concentrations of DAM and its metabolites were measured in venous ammonium-heparinized plasma obtained from 23 patients at baseline and 3 min, 10 min and 60 min after individualized heroin injection. Plasma levels of DAM, 6-acetylmorphine (6AM), morphine (M), morphine-3-β -D-glucuronide (M3G), and morphine-6-β-D-glucuronide (M6G) were assessed using high-performance liquid chromatography on a 125x2 mm i.d. Nucleosil 50 C-8 ec column with a particle size of 5 µm and a 8 x 3 mm i.d. precolumn packed with Nucleosil 120 C-8 and a particle size of 3 µm, followed by diode-array detection. Sample preparation and instrumental conditions were as described previously in detail (27). Minor optimization steps included the adjustment of the sample pH to 8.0 for the solid-phase extraction, to prevent DAM hydrolysis, and the multi-step gradient applied during the chromatographic separation.

Salivary cortisol was analyzed with a time-resolved immunoassay with fluorescence detection, as described elsewhere (8). Total cortisol concentrations were measured in serum with the Immulite 2000 Cortisol-Test (Siemens, Germany). The measurement range of the test is at 1 – 50 ug/dl; the analytical sensitivity is 0.20 ug/dl. The test shows an intraassay precision of 7.4% and an interassay precision of 9.4 %. The reference range of the concentration of cortisol depends on the time of day, with morning levels of 5 – 25 ug/dl. ACTH was measured in EDTA plasma with the ACTH Immulite-Test (Siemens, Germany). The intraassay precision was < 6.1% for concentrations > 50 pg/ml; the interassay precision was 9.4% for concentrations > 51 pg/ml. The analytical sensitivity of this test is 9 pg/ml. The recovery range of this test is 91 – 107%. The median of a study with 59 test persons in good health (male/female) performed by the manufacturer showed a value of 24 pg/ml and a 95%-reference range of n.d. – 46 pg/ml.

Interviews and self-report measures

Clinically experienced psychiatrists conducted the Structured Clinical Interview for DSM-IV for DSM-IV Axis II Disorders (SCID-II) (28) to assess the diagnosis of a comorbid personality disorder. The Heroin Craving Questionnaire (HCQ) was assessed to measure perceived craving and withdrawal level (29, 30). The State-Trait Anxiety Inventory (STAI) was administered to examine the state anxiety (31).

Statistical analyses

Statistical analyses were conducted using SPSS for Windows (version 17.0). Primary endpoints were the cortisol and ACTH levels.

A repeated-measures ANOVA was performed with the two within factors for substance (heroin vs. saline) and three time points (baseline, 20 min and 60 min after substance injection). The factor order of the substance was randomized between subjects. The random order of substance

administration, age, gender and personality disorder diagnosis were used as covariates. When within factors were significant, Tukey's post-hoc tests were performed. To protect against violations of sphericity, repeated-measures data were adjusted for within-factor degrees of freedom, using the Greenhouse-Geisser correction where appropriate.

The differences between heroin-dependent patients and healthy controls were tested with t-test for the three primary endpoints. Because of multiple comparisons, alpha was adjusted for 7 tests using the Bonferroni correction. As consequence, all statistical tests were considered significant at a two-tailed level of $p < 0.0072$.

Results

Plasma concentrations of heroin and its metabolites

Heroin (diacetylmorphine, DAM) peak plasma concentrations rose to 1005 ng/ml at 3 min after heroin administration, due to the extremely short plasma elimination half-life of the drug. At the last sampling time point, DAM (35 - 139 ng/ml) was still measurable in 3 patients. 6AM exhibited a similar time-concentration profile to that of DAM. M was detectable in all patients and at all sampling time points after administration of DAM, with a peak plasma concentration at 3 min (20 patients) and 10 min (3 patients). The decline in the plasma concentrations of M was considerably slower than for DAM and 6AM, reflecting the much longer elimination half-life for M compared to the acetylated compounds. At

3 min, 10 min and 60 min, the concentration ranges were 39 - 3885, 31 - 761, and 29 - 436 ng/ml, respectively. This indicates relatively stable plasma levels of M over a prolonged time period, with the highest inter-individual variability observed at 3 min. The M3G and M6G plasma concentrations steadily increased over the study period of 60 min, approaching a plateau at the end of the study. At the last sampling time point, the M3G concentrations were between 281 and 4432

ng/ml. Considerably lower concentrations were found for M6G, ranging between of 81 and 1099 ng/ml. The plasma profiles of heroin and its metabolites are depicted in Figure 1.

[Please here Figure 1]

Effects of heroin on self-report measures

After the application of heroin, perceived craving ($t = 5.19$, $df = 27$, $p < 0.001$) and withdrawal ($t = 4.42$, $df = 27$, $p < 0.001$) decreased significantly. After saline injection, the withdrawal level increased significantly ($t = -2.83$, $df = 27$, $p < 0.01$) and craving scores did not change. The state anxiety decreased after heroin administration ($t = 6.16$, $df = 27$, $p < 0.001$), and did not change after saline. After heroin administration, patients did not differ from healthy controls in their self-report measures.

Effects of heroin vs. saline in heroin-dependent patients

The repeated-measures ANOVA showed a significant substance (heroin vs. saline) and time interaction for ACTH ($F = 11.21$, $p = 0.001$), serum cortisol ($F = 45.59$, $p < 0.001$) and saliva cortisol concentrations ($F = 20.16$, $p < 0.001$) in heroin-dependent patients ($p < 0.001$). Moreover, there were significant time effects for ACTH ($F = 6.06$, $p = 0.014$) and serum cortisol levels ($F = 16.46$, $p < 0.001$).

According to the post-hoc tests, ACTH decreased significantly from baseline to 20 min ($F = 5.54$, $p < 0.05$) and from 20 min to 60 min after heroin administration ($F = 40.65$, $p < 0.0001$). Serum cortisol ($F = 51.50$, $p < 0.0001$) and saliva cortisol ($F = 12.99$, $p = 0.001$) decreased from baseline to 20 min after heroin administration, and serum cortisol ($F = 42.50$, $p < 0.0001$) and saliva cortisol ($F = 26.70$, $p < 0.0001$) decreased again from 20 min to 60 min after heroin administration. We

included random order of substance administration, age, gender and personality disorder diagnosis as covariates in the ANOVA. There was no significant influence on stress hormone levels.

Effects of heroin and saline in heroin-dependent patients vs. saline in healthy controls

The differences in stress hormone levels between heroin-dependent patients and healthy controls during the experiment are shown in Figures 2-4. At baseline, ACTH concentrations were significantly higher in patients than in healthy controls ($t = 2.96$, $df = 37.52$, $p = 0.005$). Sixty min after substance administration, serum cortisol and salivary cortisol did not differ between heroin-dependent patients and healthy controls when patients received heroin. ACTH concentrations were significantly lower 60 min after heroin administration in heroin-dependent-patients than in healthy controls ($t = -3.55$, $df = 46$, $p = 0.001$). When the heroin-dependent patients received saline, ACTH ($t = 4.98$, $df = 29.97$, $p < 0.0001$, serum cortisol ($t = 3.87$, $df = 46$, $p < 0.0001$), and salivary cortisol concentrations ($t = 4.01$, $df = 43.17$, $p < 0.0001$) were significantly higher than in healthy controls at the end of the experiment.

[Please here Figure 2]

[Please here Figure 3]

[Please here Figure 4]

Discussion

This study examined the acute effects of heroin on the HPA axis response in heroin-dependent patients compared to placebo and to healthy controls. We found that all stress hormones decreased in heroin-dependent patients after heroin administration. Importantly, stress hormone levels did not

differ (cortisol) or were even lower (ACTH) than those in healthy controls when they received heroin. However, during saline treatment, the stress hormone levels were higher in patients than in healthy controls. This finding highlights the acute suppressive effect of heroin on the HPA axis. Moreover, it indicates that ─ even on stable opioid maintenance treatment ─ heroin-dependent patients still show a different HPA axis response than healthy persons when they do not receive their daily opioid dosage. We have found a higher HPA axis response in heroin-dependent patients after saline injection than in healthy control persons. This finding supports previous results showing partial normalized HPA axis activity in methadone-maintained heroin-dependent patients compared to active and former heroin users (16). It could be inferred that heroin-dependent patients in HAT need to inject their daily heroin dose to suppress their HPA axis activity.

Before substance administration, hormone levels (ACTH) were elevated in patients compared to controls. This probably reflects the beginnings of withdrawal symptoms, given that the dose of heroin had not yet been administered. After heroin administration, but not after placebo, we found the expected decrease in patients' craving and withdrawal level.

The observed plasma concentrations of heroin and its metabolites confirmed the very short half-life of heroin and revealed a plateau phase, with relatively stable plasma levels of the active metabolite morphine over a prolonged time period after 10 min, underscoring this acute stress suppressive effect of heroin, even one hour after administration (32).

The clinical consequences of this heroin effect on the HPA axis have not been clear. However, it has been suggested that ─ in contrast to increased dopamine and opioid peptide function ─ increased corticotropin-releasing factor (CRF) and cortisol levels are associated with negative affects and stress like-states in drug users (33). Additionally, animal models have shown that acute stress induces drug-seeking behavior and drug self-administration after prolonged abstinence, indicating the significance of stress-like states for craving and relapse (34). In animals, this stress-induced reinforcement of drug seeking behavior appears to depend particularly on the activation of

CRH levels and the extended amygdala (35). In heroin-dependent patients, the activation of the amygdala is followed by a decrease in the activation of different brain areas, including the amygdala, after methadone (36) and buprenorphine administration (37).

We demonstrated a decrease in negative affects such as anxiety after heroin administration. This dampening effect of negative affects – including craving and withdrawal level – highlights the acute emotional regulation effect of heroin administration and may be a relevant factor in maintaining drug-taking behaviour (38). Previous studies have demonstrated the emotional regulation effect of methadone application too, but with slightly higher opioid doses (8). Moreover, studies on intravenously injected heroin may better reflect the pattern of drug use in heroin addicts.

Our findings suggest that heroin and other opioids may contribute to the normalization of impaired emotional processing and emphasize the benefits of regular opioid substitution for heroin-dependent patients (39).

We conclude that HPA axis activity and negative affects can be significantly suppressed by regular opioid administration in heroin-dependent patients, which may also prevent later illegal drug use and relapse.

Our patients were recruited from a population which mainly consisted of individuals with long-standing polysubstance use. Although this problem is virtually inevitable when chronic heroin-dependent individuals are examined, it may have biased the results. The findings may thus not apply to all groups of heroin users and maintenance patients. We examined the effects of heroin in a controlled study design that is only possible in a country that has heroin-assisted treatment programs. We did not have a completely balanced study design. However, there was no significant influence of the random order of the injected substance in the patient group.

These limitations notwithstanding, we think our results retain significant clinical implications. They establish that heroin suppresses the HPA axis response and may protect stress-sensitive heroin-dependent patients against their heightened stress response.

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Table 1: Socio-demographic and diagnostic characteristics of the study sample. Experimental group (patients) *n* = 28, control group (healthy persons) *n* = 20.

Measurements	Mean (*SD*) Experimental group	Control group	p-value
Age	41.3 (6.6)	40.3 (10.9)	.718
Male gender (%)	19 (67.9)	14 (70.0)	.875
Partnership (%)	9 (32.1)	15 (75.0)	.003
Employment (%)	11 (39.3)	20 (100.0)	.000
Disability (%)	9 (32.1)	0 (0.0)	.005
Doses of DAM (mg/day)	318.6 (131.7)	-	
Methadone maintenance (%)	13 (46.4)	-	
Doses of methadone (mg/day)	13.4 (17.4)	-	
Duration of dependence (years)	20.8 (6.6)	-	
Age at the first-time heroin use (years)	19.0 (3.4)	-	
Duration of opioid maintenance (years)	6.7 (4.5)	-	
Substance abuse:			
- tobacco (%)	28 (100.0)	20 (100.0)	1.000
- number of cigarettes/day	21.0 (9.1)	11.5 (8.2)	.001
- cocaine (%)	15 (53.6)	-	
- cannabis (%)	8 (28.6)	-	

Note: *SD* = Standard deviation.

Table 2: Effects of the interaction between the substance (DAM/placebo) and the measurement time on the stress hormone distributions.

Source	*SS*	*df*	*MS*	*F*	*p-value*
ACTH					
substance	18191.06	1.00	18191.06	8.75	.007
substance x group	10378.19	1.00	10378.19	4.99	.034
error (substance)	54075.96	26.00	2079.85		
time	8727.18	1.28	6822.93	8.64	.003
time x group	1473.97	2.00	736.99	1.46	.242
error (time)	26267.26	33.26	789.84		
substance x time	7817.41	1.26	6222.89	8.04	.005
substance x time x group	187.78	2.00	93.89	0.19	.825
error (substance x time)	25287.99	32.66	774.23		
Cortisol serum					
substance	273430.98	1.00	273430.98	7.08	.013
substance x group	264945.12	1.00	264945.12	6.86	.015
error (substance)	1004903.26	26.00	38650.13		
time	330203.36	1.56	212379.78	29.33	.000
time x group	24674.72	2.00	12337.36	2.19	.122
error (time)	292671.56	40.42	7240.01		
substance x time	415104.72	1.41	293797.10	37.33	.000
substance x time x group	10751.19	2.00	5375.59	0.97	.387
error (substance x time)	289112.73	36.74	7870.16		
Cortisol saliva					
substance	7912.05	1.00	7912.05	8.14	.008
substance x group	8016.98	1.00	8016.98	8.25	.008
error (substance)	25276.02	26.00	972.15		
time	834.43	2.00	417.22	1.00	.376
time x group	2699.93	2.00	1349.97	3.22	.048
error (time)	21774.80	52.00	418.75		
substance x time	7662.70	2.00	3831.35	16.99	.000
substance x time x group	1468.06	2.00	734.03	3.26	.047
error (substance x time)	11726.02	52.00	225.50		

Note: *SS* = Sum of Squares; *df* = degrees of freedom; *MS* = Mean Square, x = Interaction.

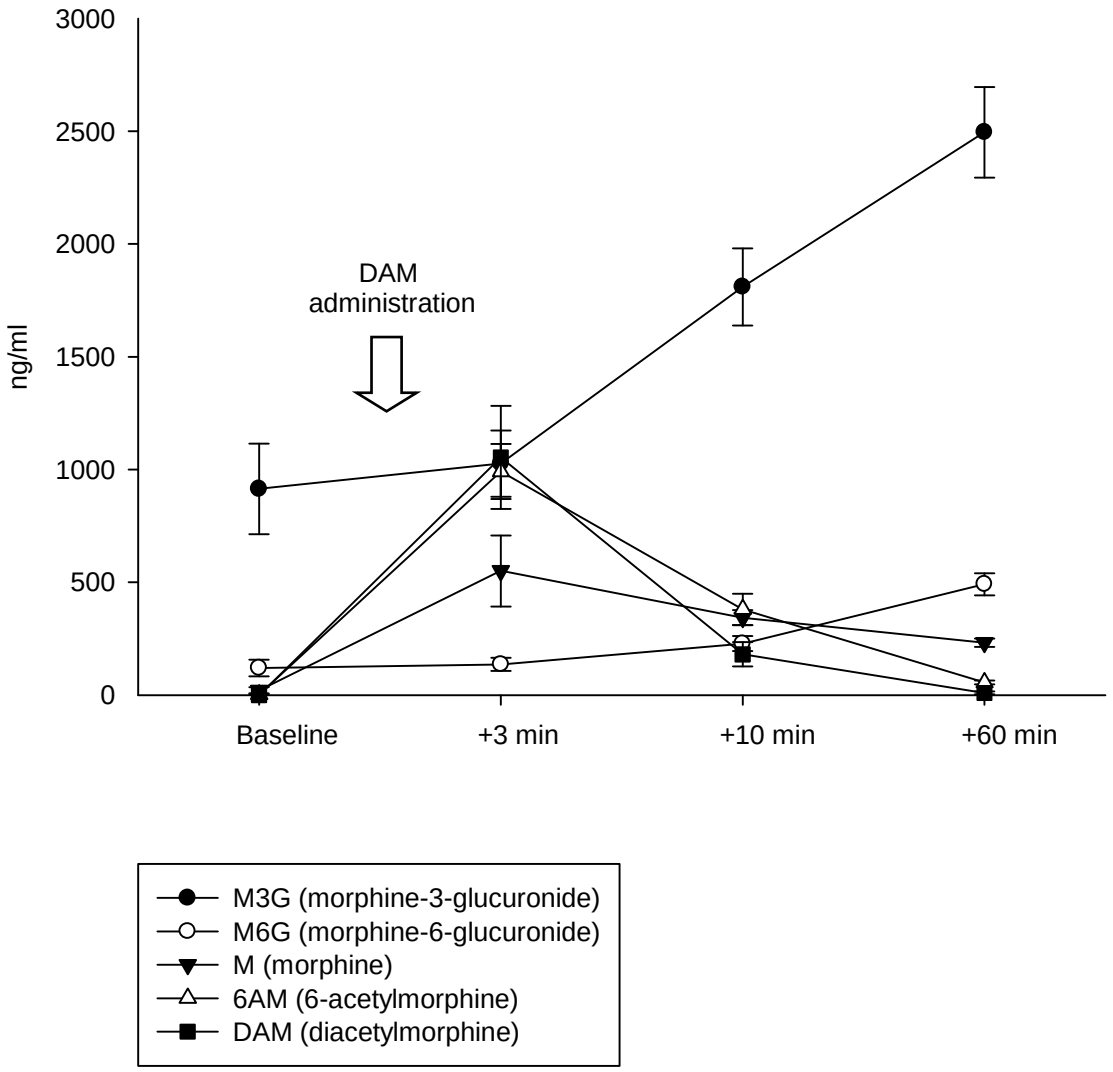

Figure 1: Plasma concentrations of diacetylmorphine (DAM) and its metabolites in diacetylmorphine (heroin)-maintained patients before and after diacetylmorphine (heroin) injection. Means and standard errors are displayed.

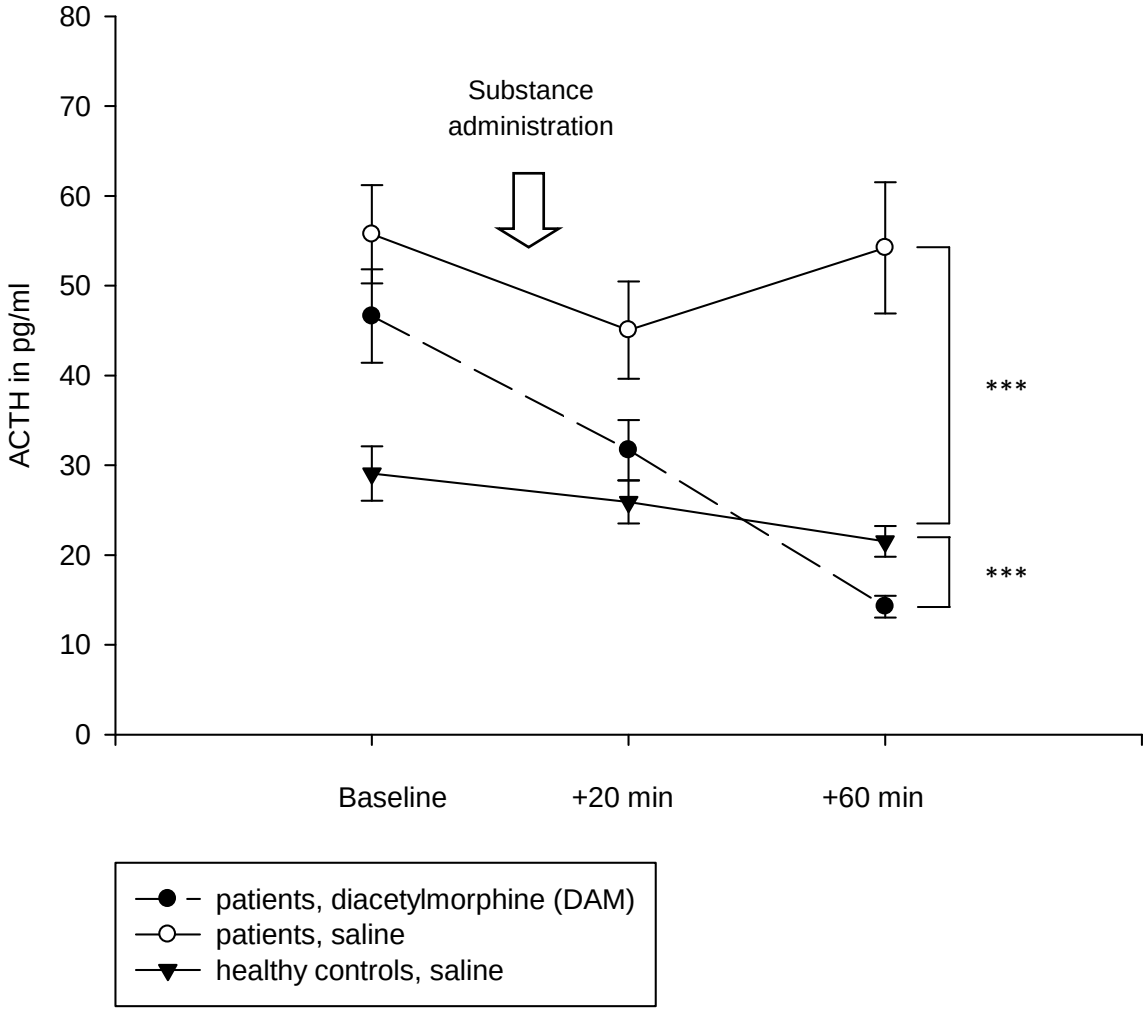

Figure 2: ACTH concentration in diacetylmorphine (heroin)-maintained patients after diacetylmorphine (heroin) or placebo (saline) injection and healthy controls after placebo (saline) injection. Means and standard errors are displayed. *** = $p < 0.001$.

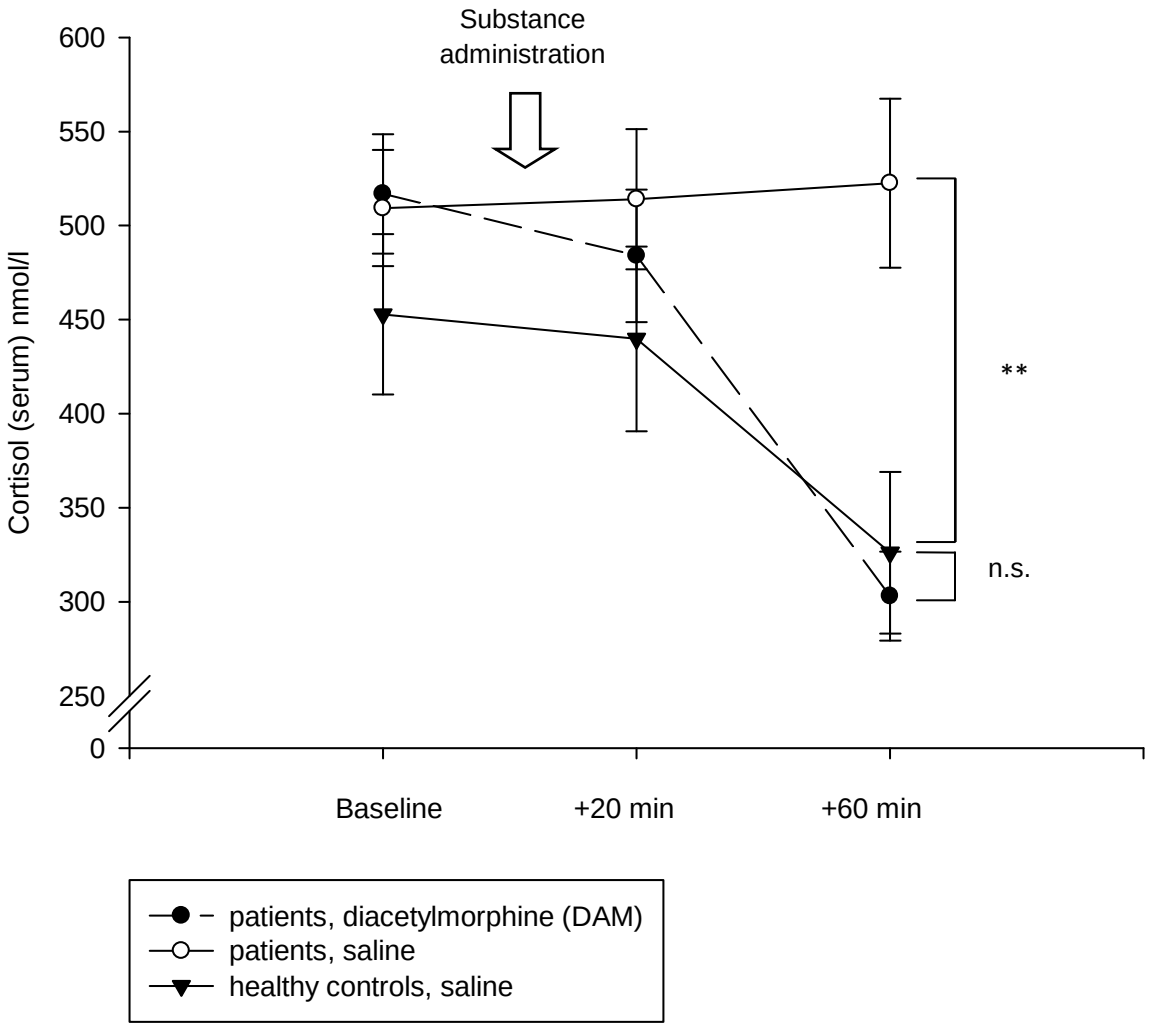

Figure 3: Serum cortisol concentration in diacetylmorphine (heroin)-maintained patients after diacetylmorphine (heroin) or placebo injection and healthy controls after placebo (saline) injection. Means and standard errors are displayed. ** = $p < 0.01$.

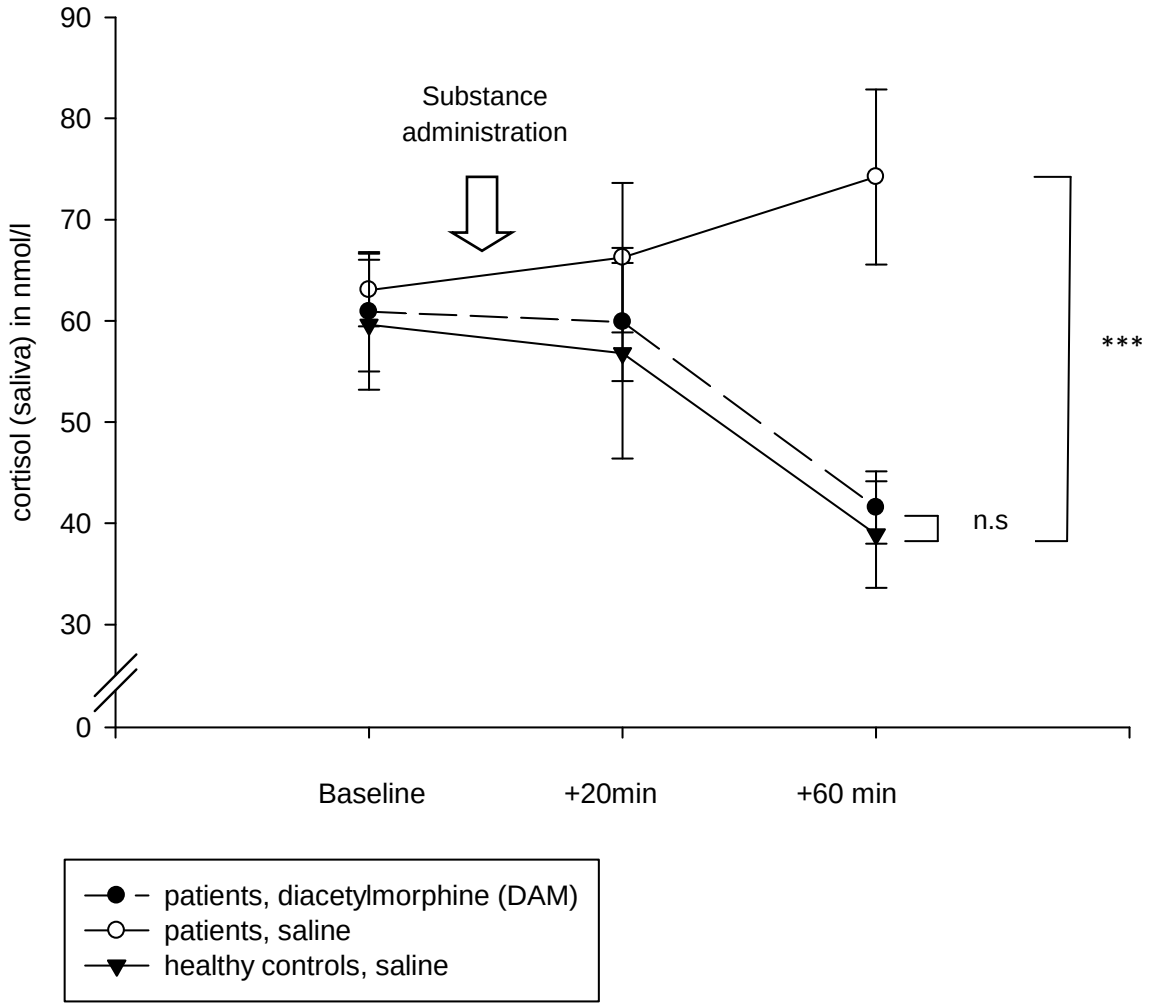

Figure 4: Saliva cortisol concentration in diacetylmorphine (heroin)-maintained patients after diacetylmorphine (heroin) or placebo injection and healthy controls after placebo (saline) injection. Means and standard errors are displayed. *** = $p < 0.001$.